Mechanistic Data, Cellular Pathways and Cancer Classification

Dr. Christopher J. Portier

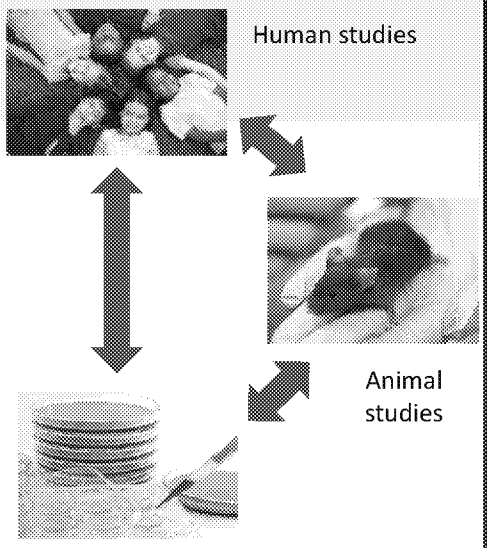
39th Annual Winter Toxicology Forum

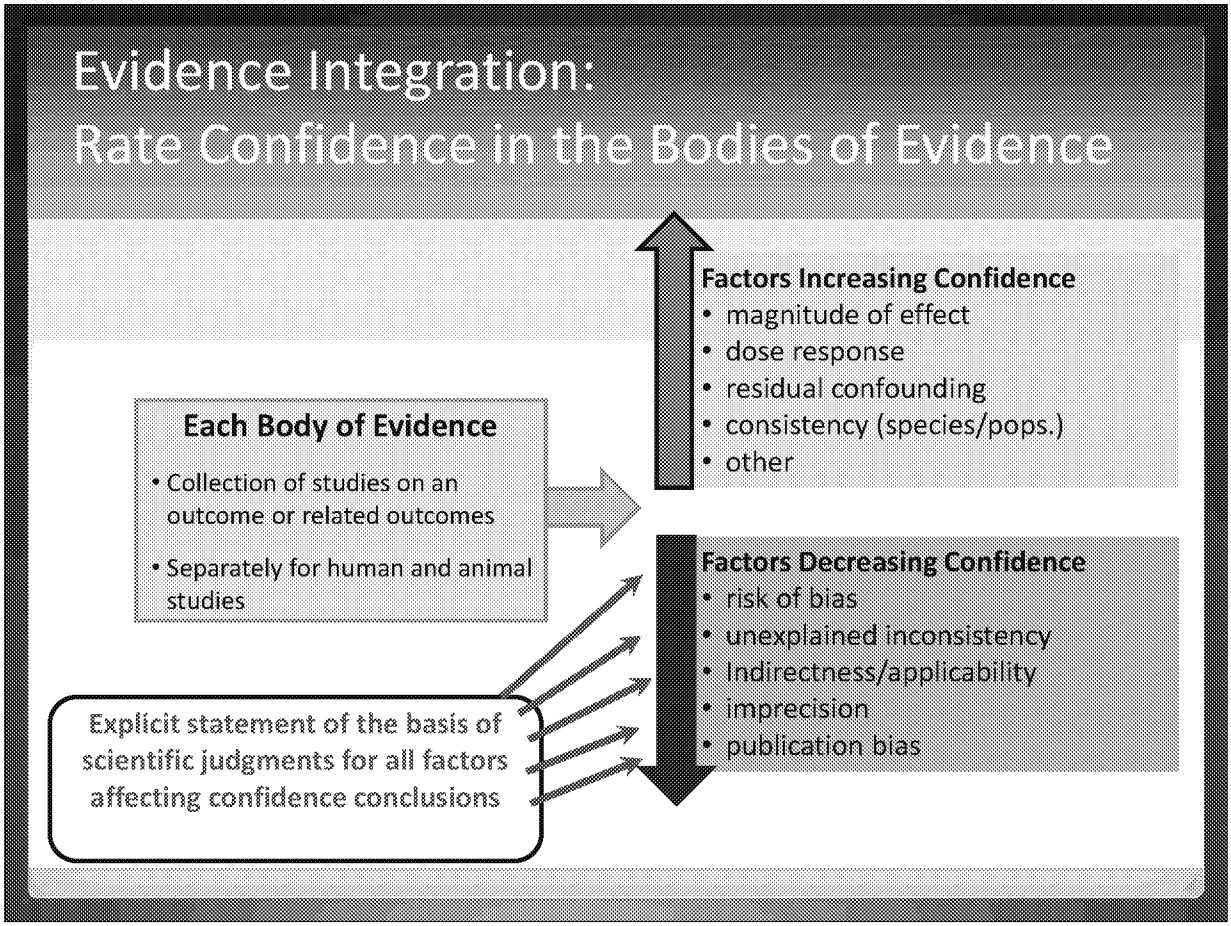
Washington, DC

January, 2015

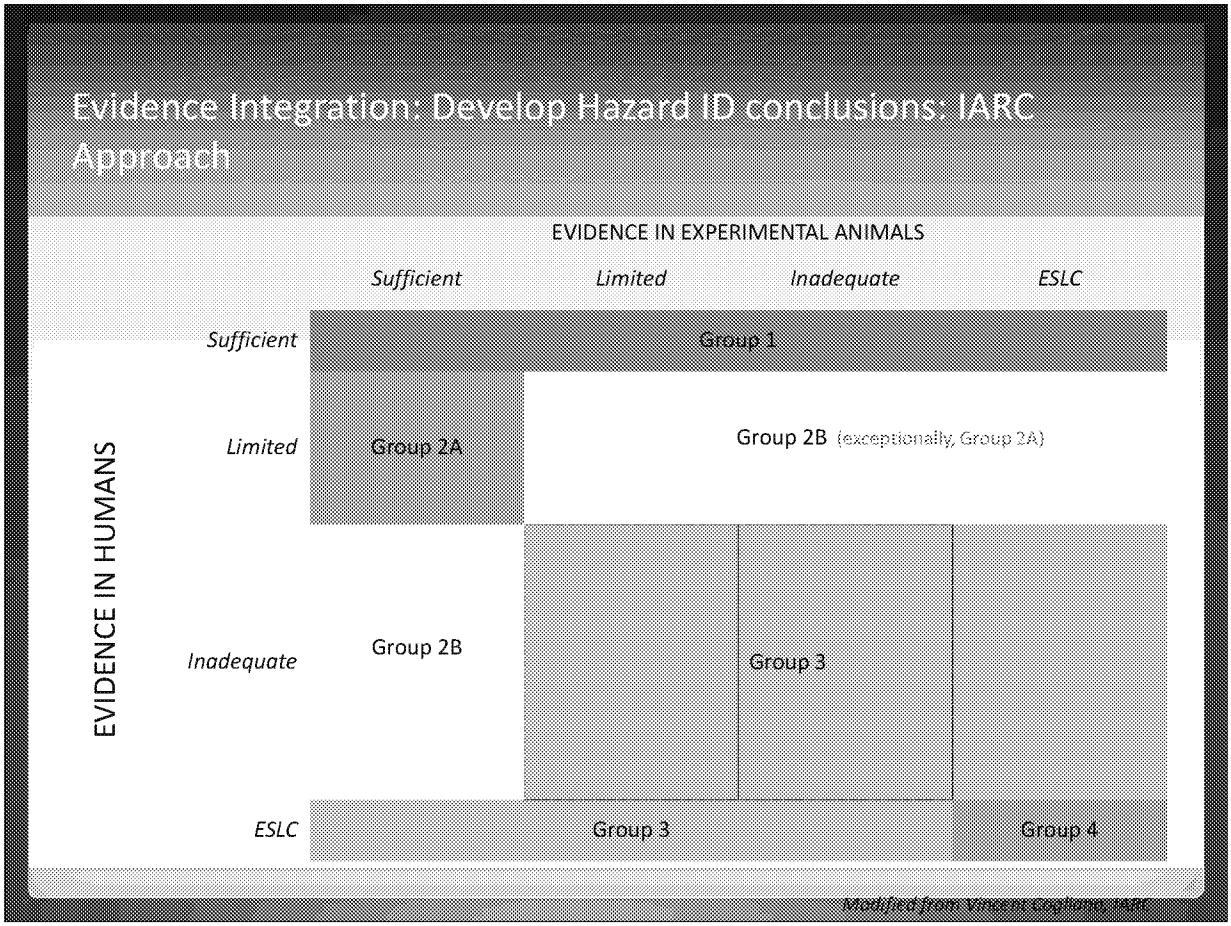
Requirements for Systematic Review Methods in Environmental Health

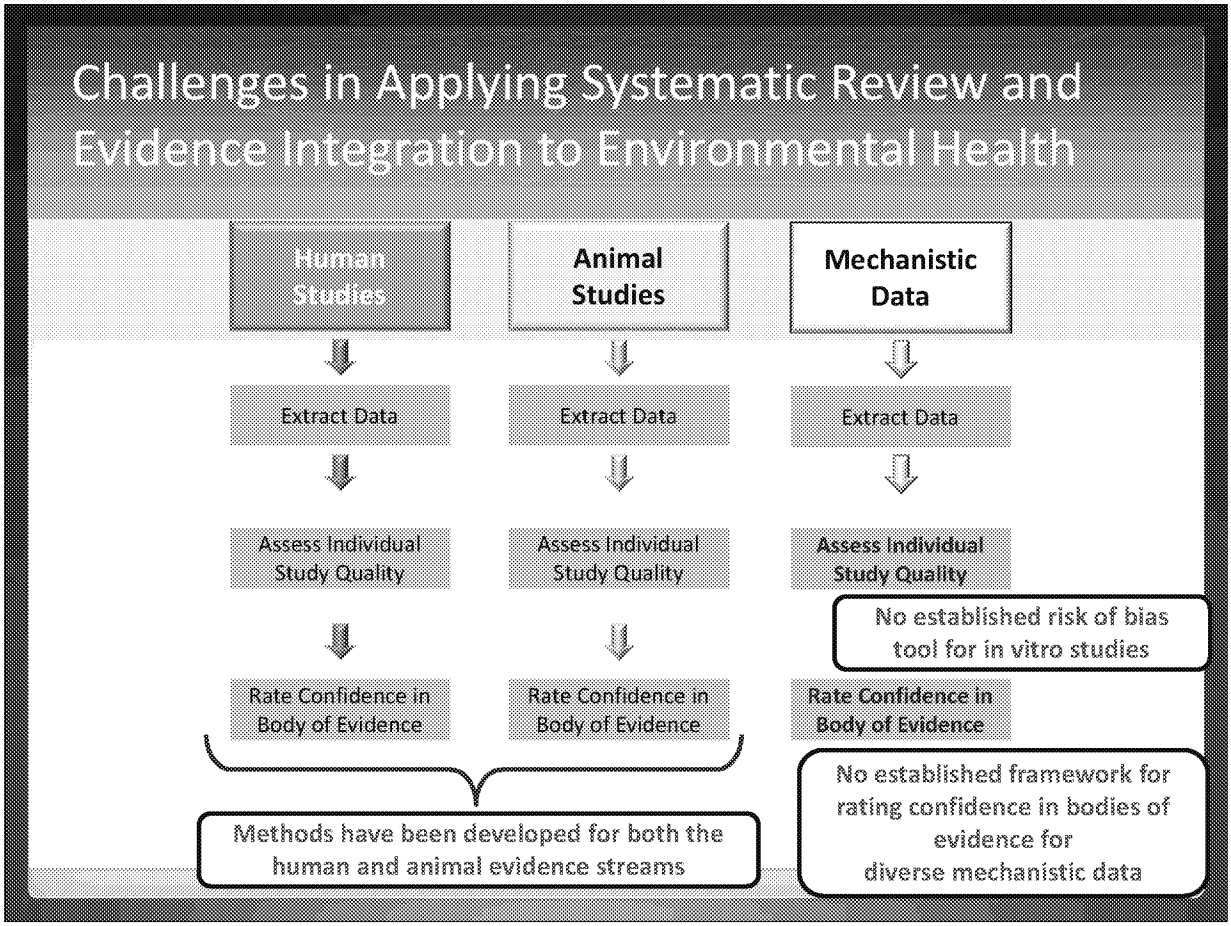
- Framework to increase transparency and objectivity
- Address the breadth of relevant data
  - Wide range of human study designs
  - Animal studies
  - Mechanistic studies (*in vitro* and other relevant data)
- Procedure to integrate evidence streams





Allows us to evaluate as a group for the same outcome (or set of related outcomes)


Evidence Integration: Develop Hazard ID conclusions: IARC Approach
EVIDENCE IN EXPERIMENTAL ANIMALS
Sufficient
Limited
Inadequate
ESLC
EVIDENCE IN HUMANS
Sufficient
Limited
Inadequate
ESLC
Group 1
Group 2A
Group 2B (exceptionally, Group 2A)
Group 2B
Group 3
Group 3
Group 4
Modified from Vincent Cogliano, IARC

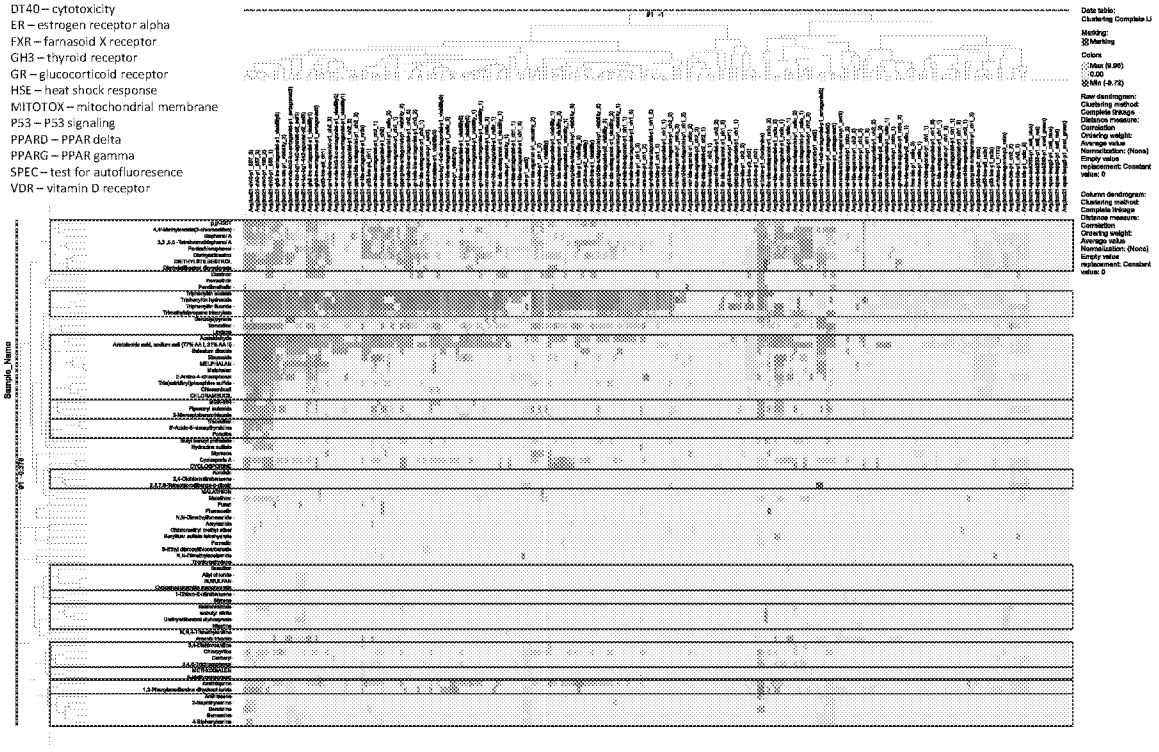

Challenges in Applying Systematic Review and Evidence Integration to Environmental Health
Human Studies
Animal Studies
Mechanistic Data
Extract Data
Extract Data
Extract Data
Assess Individual Study Quality
Assess Individual Study Quality
Assess Individual Study Quality
No established risk of bias tool for in vitro studies
Rate Confidence in Body of Evidence
Rate Confidence in Body of Evidence
Rate Confidence in Body of Evidence
Methods have been developed for both the human and animal evidence streams
No established framework for rating confidence in bodies of evidence for diverse mechanistic data

Use of Mechanistic Data

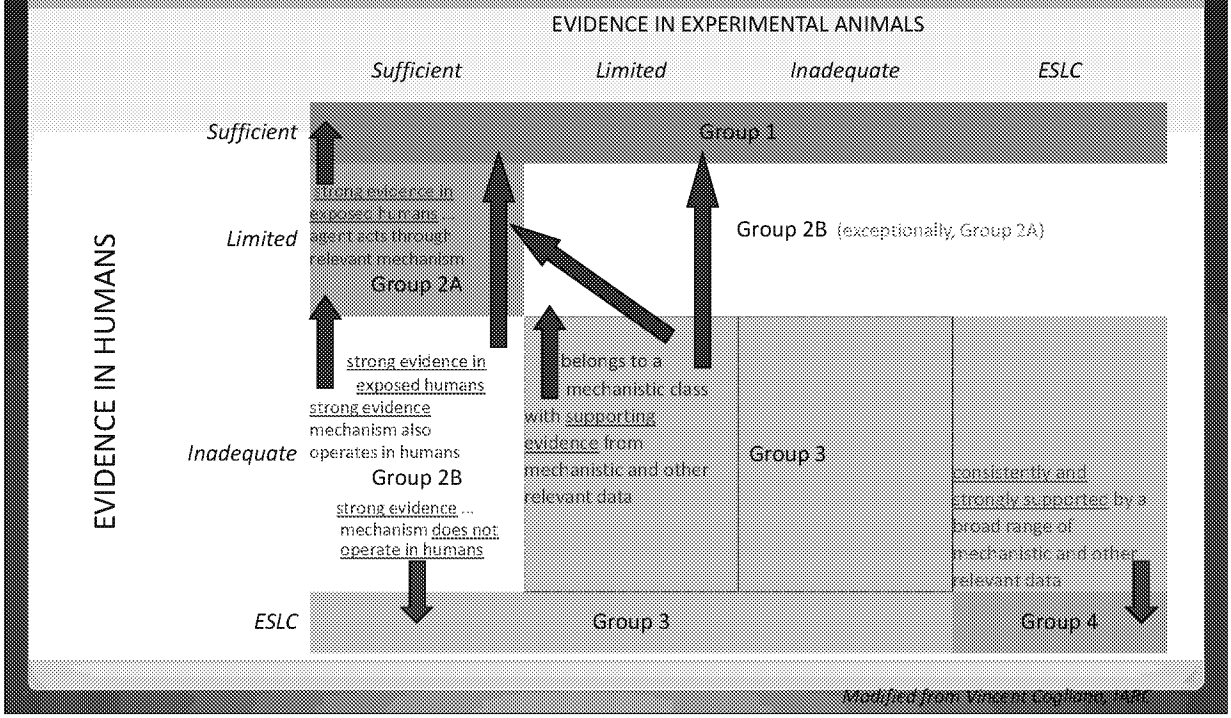
- Setting Priorities
- Evaluating Literature
- Identifying Hazards
- Communication

Heat Map of Group 1 and Advisory Group Chemicals that are in Tox21-v2 Database

AHR – Ah receptor
AR – androgen receptor
ARE – antioxidant response element
Aromatase – aromatase inhibitors
DT40 – cytotoxicity
ER – estrogen receptor alpha
FXR – farnasoid X receptor
GH3 – thyroid receptor
GR – glucocorticoid receptor
HSE – heat shock response
MITOTOX – mitochondrial membrane
P53 – P53 signaling
PPARD – PPAR delta
PPARG – PPAR gamma
SPEC – test for autofluoresence
VDR – vitamin D receptor

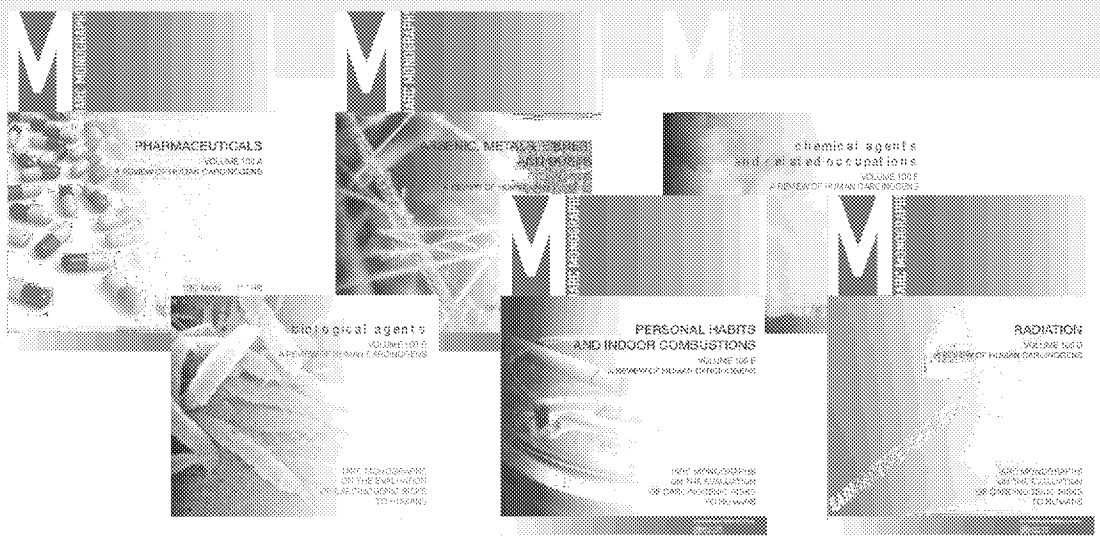


Mechanistic data can be pivotal when the human data are not conclusive - IARC



Modified from Vincent Cogliano, IARC

Monograph 100 A-F Review



Key Characteristics of Carcinogens

- What are they?
  - Properties commonly shown by known human carcinogens
- How are they used?
  - Classify evidence into groups of endpoints focused around a common characteristic
    - Enables systematic evaluation of the literature
  - Convenient means to search literature for mechanistic data related to carcinogenesis
  - Communication of mechanistic conclusions from a review
- What are they NOT?
  - Mechanisms of carcinogenesis
  - Adverse outcome pathways

Key Characteristics of Carcinogens

- Electrophilicty and Metabolic activity
  - electron-seeking molecules that commonly form addition products, commonly referred to as adducts
  - binds with DNA, RNA and proteins
- Genotoxicity
  - induces DNA damage
- Altered repair and genomic instability
  - alters DNA replication fidelity

Key Characteristics of Carcinogens
(continued)

- Chronic inflammation
  - disrupts local tissue homeostasis and alters cell signaling
- Oxidative stress
  - creates an imbalance in reactive oxygen formation and/or alters their detoxification
- Receptor-mediated
  - Acts as ligand via nuclear and/or cell-surface and/or intracellular receptors

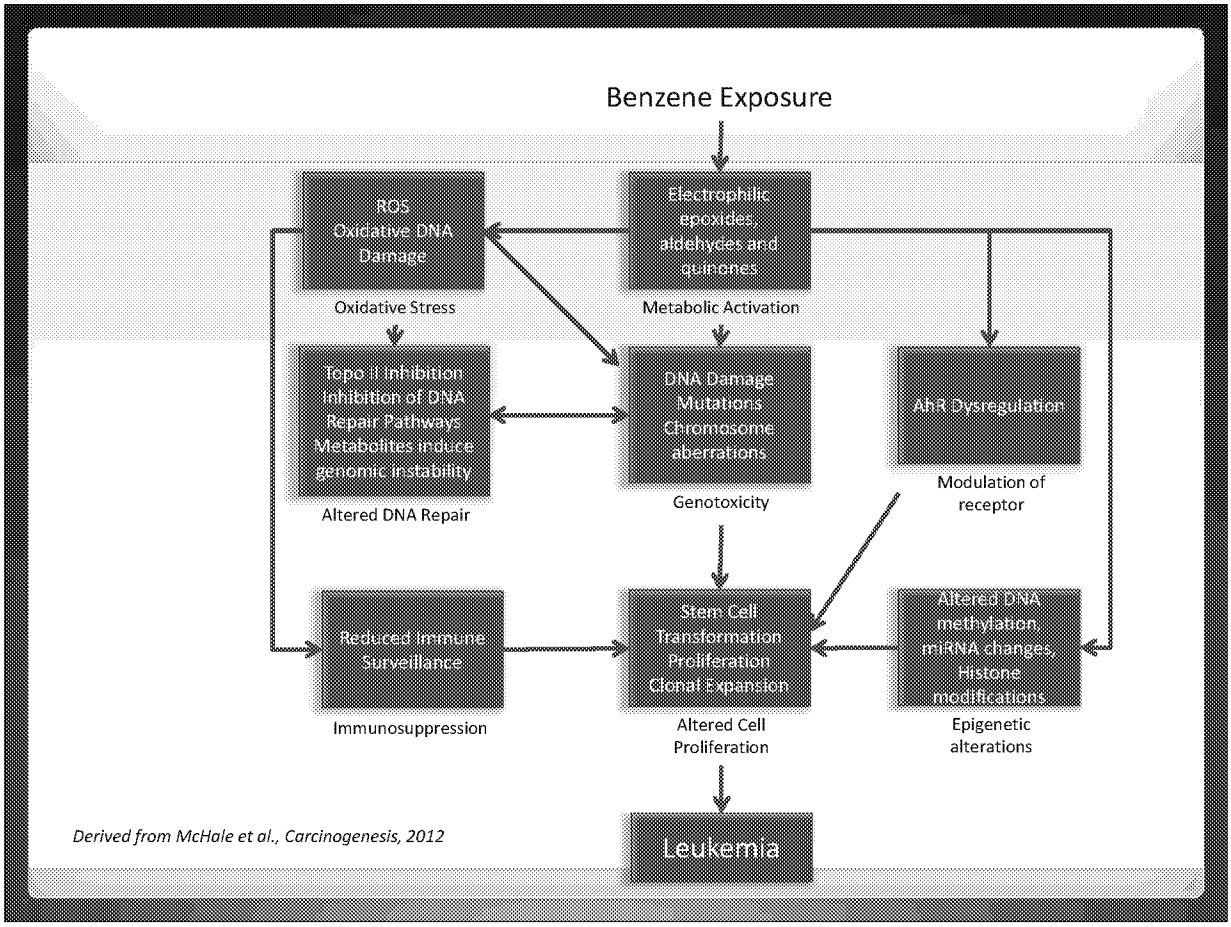
Key Characteristics of Carcinogens
(continued)

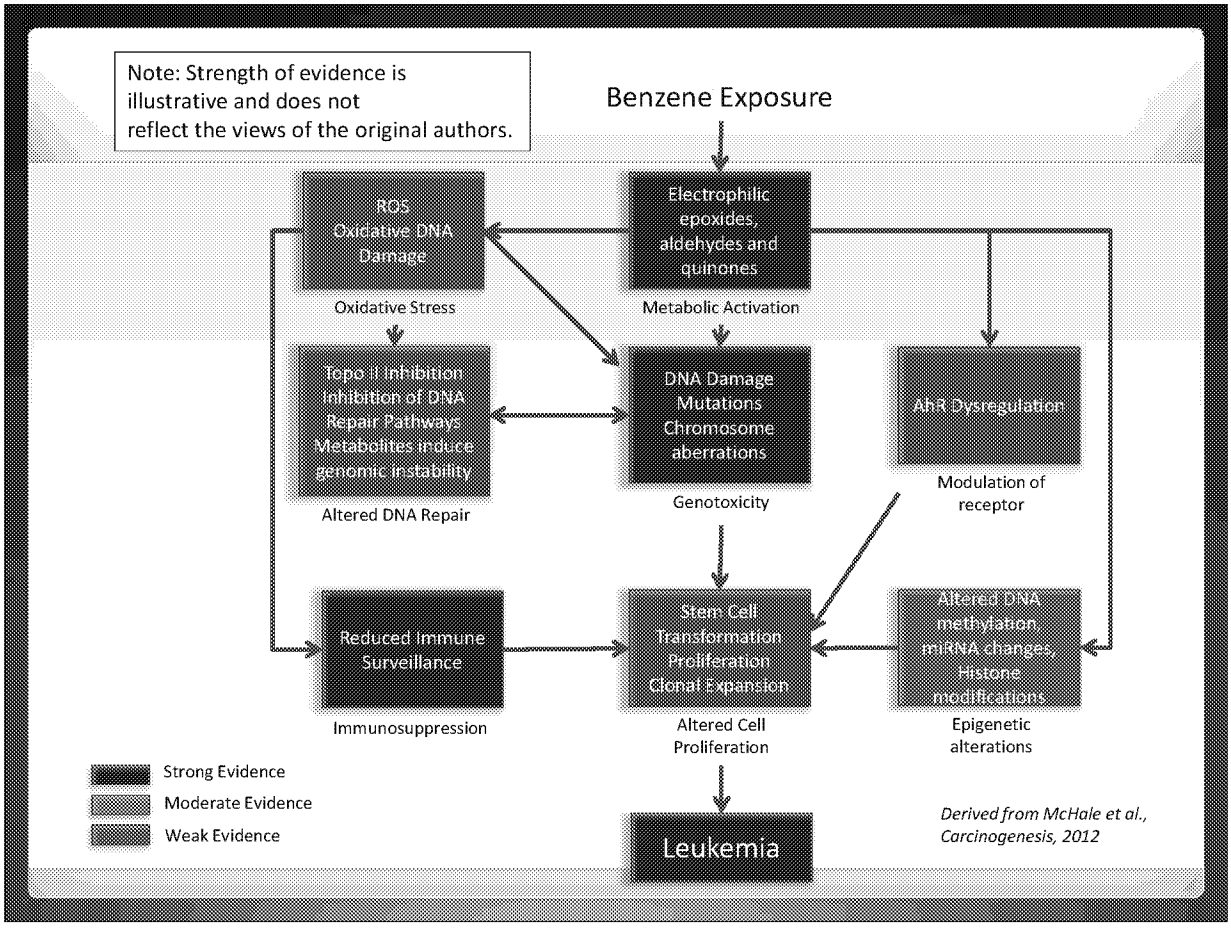
- Altered cellular proliferation and/or death
  - alterations in cellular replication and/or cell-cycle control resulting in escape from growth control or mutations or inflammation
- Immunosuppression
  - reduces the capacity of the immune system to respond effectively to antigens on tumour cells

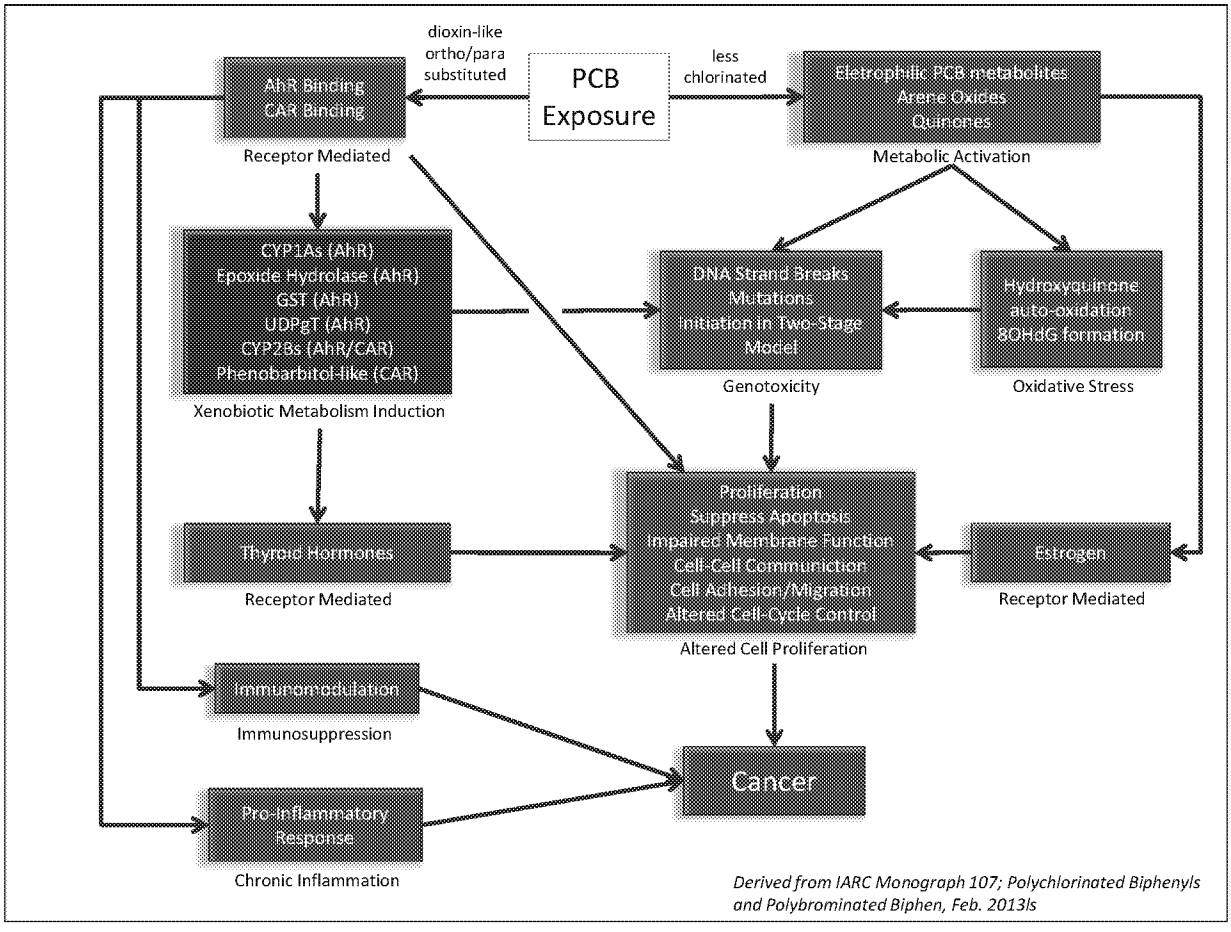
Key Characteristics of Carcinogens
(continued)

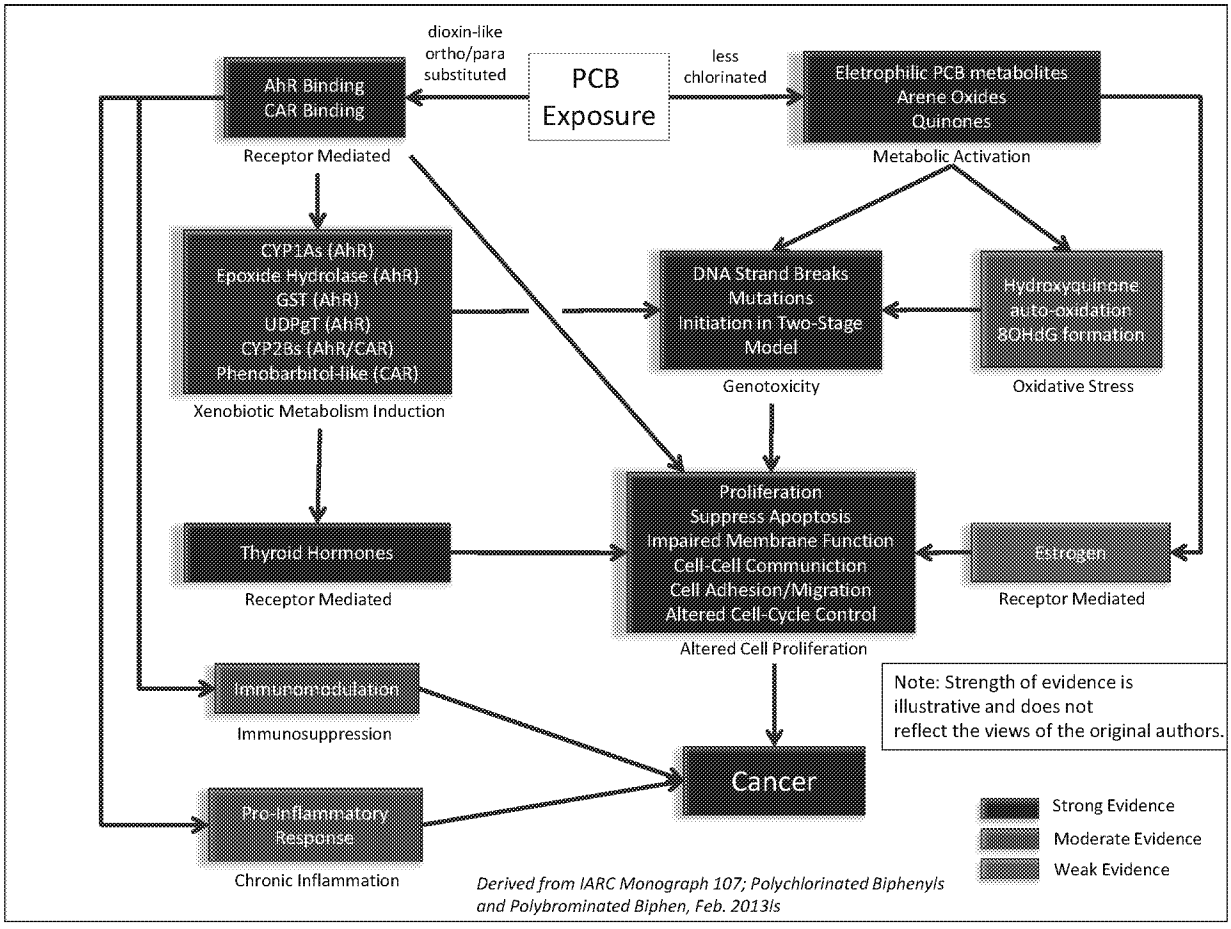
- Epigenetic alterations
  - Induces stable and heritable changes in gene expression and chromatin organization that are independent of the DNA sequence itself
- Immortalization
  - DNA and RNA viruses that produce viral-encoded oncoproteins targeting the key cellular proteins that regulate cell growth

Characteristic[1]	Example relevant evidence	Commonly Linked Characteristics[2]
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.	2,3,4,7,8,9
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).	1,3,4,5,10
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)	1,2,4,6,7,9,10
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNAs	1,6,10
5. Induces Oxidative Stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)	2,6,8,10
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production	3,4,5,7,8,10
7. Is Immunosuppressive	Decreased immunosurveillance, immune system dysfunction	1,3,6,8,9
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)	1,5,6,7,10
9. Causes Immortalization	Inhibition of senescence, cell transformation	1,3,7,10
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis	2,3,4,5,6,8,9


Benzene Exposure
ROS
Oxidative DNA Damage
Oxidative Stress
Electrophilic epoxides, aldehydes and quinones
Metabolic Activation
Topo II inhibition
Inhibition of DNA Repair Pathways
Metabolites induce genomic instability
Altered DNA Repair
DNA Damage
Mutations
Chromosome aberrations
Genotoxicity
AhR Dysregulation
Modulation of receptor
Reduced Immune Surveillance
Immunosuppression
Stem Cell Transformation
Proliferation
Clonal Expansion
Altered Cell Proliferation
Altered DNA methylation
miRNA changes
Histone modifications
Epigenetic alterations
Leukemia
Derived from McHale et al., Carcinogenesis, 2012


Note: Strength of evidence is illustrative and does not reflect the views of the original authors.
Benzene Exposure
ROS Oxidative DNA Damage
Electrophilic epoxides, aldehydes and quinones
Oxidative Stress
Metabolic Activation
Topo II inhibition Inhibition of DNA Repair Pathways Metabolites induce genomic instability
DNA Damage Mutations Chromosome aberrations
AhR Dysregulation
Altered DNA Repair
Genotoxicity
Modulation of receptor
Reduced Immune Surveillance
Stem Cell Transformation Proliferation Clonal Expansion
Altered DNA methylation miRNA changes, Histone modifications
Immunosuppression
Altered Cell Proliferation
Epigenetic alterations
Strong Evidence
Moderate Evidence
Weak Evidence
Leukemia
Derived from McHale et al., Carcinogenesis, 2012


dioxin-like
ortho/para
substituted
PCB
Exposure
less
chlorinated
AhR Binding
CAR Binding
Receptor Mediated
Eletrophilic PCB metabolites
Arene Oxides
Quinones
Metabolic Activation
CYP1As (AhR)
Epoxide Hydrolase (AhR)
GST (AhR)
UDPgT (AhR)
CYP2Bs (AhR/CAR)
Phenobarbitol-like (CAR)
Xenobiotic Metabolism Induction
DNA Strand Breaks
Mutations
Initiation in Two-Stage
Model
Genotoxicity
Hydroxyquinone
auto-oxidation
8OHdG formation
Oxidative Stress
Proliferation
Suppress Apoptosis
Impaired Membrane Function
Cell-Cell Communiction
Cell Achesion/Migration
Altered Cell-Cycle Control
Altered Cell Proliferation
Thyroid Hormones
Receptor Mediated
Estrogen
Receptor Mediated
Immunomodulation
Immunosuppression
Cancer
Pro-Inflammatory
Response
Chronic Inflammation
Derived from IARC Monograph 107; Polychlorinated Biphenyls
and Polybrominated Biphen, Feb. 2013ls


dioxin-like
ortho/para
substituted
PCB
Exposure
less
chlorinated
AhR Binding
CAR Binding
Receptor Mediated
Eletrophilic PCB metabolites
Arene Oxides
Quinones
Metabolic Activation
CYP1As (AhR)
Epoxide Hydrolase (AhR)
GST (AhR)
UDPgT (AhR)
CYP2Bs (AhR/CAR)
Phenobarbitol-like (CAR)
Xenobiotic Metabolism Induction
DNA Strand Breaks
Mutations
Initiation in Two-Stage
Model
Genotoxicity
Hydroxyquinone
auto-oxidation
8OHdG formation
Oxidative Stress
Proliferation
Suppress Apoptosis
Impaired Membrane Function
Cell-Cell Communiction
Cell Adhesion/Migration
Altered Cell-Cycle Control
Altered Cell Proliferation
Thyroid Hormones
Receptor Mediated
Estrogen
Receptor Mediated
Immunomodulation
Immunosuppression
Cancer
Pro-Inflammatory
Response
Chronic Inflammation
Note: Strength of evidence is
illustrative and does not
reflect the views of the original authors.
Strong Evidence
Moderate Evidence
Weak Evidence
Derived from IARC Monograph 107; Polychlorinated Biphenyls
and Polybrominated Biphen, Feb. 2013ls

Moving Forward

- How does Tox21 and ToxCast data fit into this framework?
- Genomics, Proteomics, etc.?
  - Pathway-driven analyses
  - Other?
- Do we need formal rules for what constitutes "Strong"...?
- Systematic Review of Mechanistic Data
  - Magnitude of the problem
  - Complexity of the different data types
- How does this relate to AOP and existing regulatory structures for Hazard ID?
  - Quantitative risk assessment implications?